REMARKS

I. Status of the Application

Claims 1-46 are presently pending in the application. Claims 1-3, 5, 9, 14, 18, 29-33, 35-37, 39 and 43 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al., U.S. Patent No. 6,203,987, in view of Eberwine, U.S. Patent No. 5,514,545. Claims 12, 15-17, 19-22, 24, 27, 28 and 34 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Lockhart et al., *Nature Biotechnology* 14:1675 (1996). Claims 4, 7, 8, 10, 11, 13 and 41 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Hampson et al., U.S. Patent No. 6,066,457. Claim 6 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Van Ness et al., U.S. Patent No. 6,248,521. Claims 23, 45 and 46 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Hampson et al. and North et al., U.S. Patent No. 6,114,502. Claims 38, 40 and 42 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Dale, U.S. Patent No. 6,087,112. Claim 44 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Hampson et al. and North et al. and Dale. Claims 25 and 26 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Scanlon, U.S. Patent No. 5,814,489.

Applicants respectfully request entry and consideration of the foregoing remarks, which are intended to place this case in condition for allowance.

II. Claims 1-3, 5, 9, 14, 18, 29-33, 35-37, 39 and 43 Are Patentable Over Friend et al. in View f Eberwine

At page 2, section 1 of the instant Office Action, claims 1-3, 5, 9, 14, 18, 29-33, 35-37, 39 and 43 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Friend et al., U.S. Patent No. 6,203,987, in view of Eberwine, U.S. patent No. 5,514,545. The Examiner asserts that Eberwine explicitly teaches deriving nucleic acids from a single cell to characterize cell identity or physiological state, and that it teaches the detailed process of deriving single cells through pipetting. The Examiner asserts that one of skill in the art would have been motivated to combine Eberwine's teaching of derivation of nucleic acids from single cells with Friend et al.'s method of analysis in order to compare drug treatment effect on cells. The Examiner concludes that it would have been *prima facie* obvious to apply Eberwine's technique to Friend et al.'s expression array in order to successfully derive nucleic acids from single cells and examine the expression profile of single cell interactions. Applicants respectfully traverse the Examiner's rejection.

The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. Furthermore, one of skill in the art would need to understand that the modification to arrive at the claimed subject matter can be made with ***a reasonable expectation of success***. Based on the teachings of Friend et al. and Eberwine as a whole, one of skill in the art would not have a reasonable expectation of success using the techniques of Eberwine to modify the teachings of Friend et al.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. Friend et al. is not directed to analyzing gene expression in less than 1000 cells, as claimed by Applicants. Friend et al. is directed to enhanced computational methods for analyzing datasets generated from biological samples. Although Friend et al. states at col. 5 lines

34-36 that the term "biological sample" is broadly defined to include any cell, tissue, organ or multicellular organism, this reference lacks any teaching of any methods for the creation of datasets generated from single cells or from fewer than 1000 cells.

In order to analyze gene expression in a single cell or in fewer than 1000 cells, one would first need to isolate the single cell or a collection of less than 1000 cells from other cells. Applicants teach that individual cells may be isolated through the use of techniques such as microdissection, cell sorting, and serial dilution (specification, page 12, line 32 to page 13, line 2). Applicants provide working examples of single neuron isolation by microdissection (page 24), and derive single cells from olfactory epithelium or vomeronasal epithelium (page 33), and from neuronal, neuronal precursor and embryonic cells (page 34).

In contrast, Friend provides no teaching of how to isolate cDNA from a single cell. Friend provides only one example where RNA is extracted from cells. In col. 41 lines 34-39, Friend grows yeasts cells to an OD_{600} of 1.0 (±0.2) and then breaks the cells to obtain the total RNA. Applicants respectfully submit that the amount of yeast cells grown by Friend based on the optical density is several orders of magnitude larger than the less than 1000 cells of claim 1. Applicants were the first to analyze the gene expression characteristics of fewer than 1000 cells and even of a single cell. Applicants' invention is a very significant advance over gene expression methods using microarrays such as Friend that use cells on the order of a million or more from which to extract cellular material.

Furthermore, Friend et al. is concerned with the generation of microarrays using ***yeast*** genetic material. Friend teaches that "in a preferred embodiment, the invention is carried out using a yeast, with *Saccharomyces cerevisiae* most preferred" (column 31, lines 34-39). In fact, ***all*** of the working examples, which teach protocols such as RNA preparation (example 1) and

classification of drug activity (example 3), ***are directed to S. cerevisiae***. Figures 4B-4D illustrate three main clusters of yeast genes, Figures 6 and 7 show clustering trees from various experiments using yeast cells, Figures 8A-8E depict amplitudes of individual elements of the projected yeast profile, and Figure 9 shows yeast gene responses to the drug FK506 (column 4, Brief Description of the Drawings, and Examples 1-3). Friend et al. provides no experimentation or data regarding any cell type other than yeast, and does not teach monitoring genetic expression of a single cell or from fewer than 1000 cells, as claimed by Applicants.

In contrast to the teachings of Friend et al., Eberwine is specifically concerned with investigating mRNA levels in individual cells from the ***central nervous system*** because such cells have been studied electrophysiologically as single cells, but biochemically only as complex neural systems (column 1, lines 33-58). The Eberwine reference is directed to determining relative amounts of mRNAs that encode specific proteins of individual brain cells and to demonstrating an "expression profile" of the individual cells (column 3, lines 58-62; column 4, lines 11-13; and column 9, page 52-65).

Eberwine teaches a method of characterizing individual cells by concurrently monitoring cell currents and dialyzing primers and enzyme into an ***individual live cell*** using a patch-clamp recording pipette to produce cDNA inside the live cell (column 3, lines 3-11 and column 4, lines 1-33). The Eberwine reference teaches the use of their patch-clamp pipette technique for cDNA synthesis in various neuronal and glial cells such as striatum cells, NG108-15 cells, hippocampal cells, and CA1 pyramidal cells (column 8, lines 17-20 and 45-47; column 10, lines 28-31; column 14, lines 30-32). Eberwine provides no teachings that their methods are suitable for yeast cells, and one of skill in the art would have no reasonable expectation of success of substituting the methods of Eberwine to produce cDNA inside an individual yeast cell.

A major difference between yeast cells and mammalian cells such as the neuronal and glial cells taught by Eberwine is that the plasma membrane of yeast cells is encased in a ***cell wall***. Because mammalian cells lack cell walls and contain only a plasma membrane, it is possible for small molecules to traverse the plasma membrane and enter the cytoplasm of the cell. Indeed, Eberwine teaches the dialysis of oligonucleotides and reverse transcriptase across the plasma membrane of neuronal cells (column 4, lines 11-18). However, successfully adding reverse transcription reagents into a live ***yeast*** cell using the patch-clamp pipette technique of Eberwine would likely be impossible or at least very complicated, requiring more than routine experimentation, and would certainly be beyond the teachings of Eberwine. The cell wall of yeast is "thick and ***largely impenetrable***" and ***prevents the introduction of externally added DNA*** without the use of wall degrading enzymes (Attachment A, Molecular Biology of the Gene, 4th ed., emphasis added). Thus, one could not reasonably expect to place a patch-clamp pipette on the cell wall of a yeast cell and successfully dialyze oligonucleotides and reverse transcriptase through the thick, impermeable cell wall and into the yeast cytoplasm based on the teachings of Eberwine. The Examiner provides no evidence that this could be achieved.

The methods of analyzing yeast cells and neuronal cells set forth in the references cited by the Examiner employ very different procedures and protocols and reflect the fact that these cell types are not the same. For instance, in order to obtain genetic material from yeast cells, Friend et al. prepares total and poly(A)+ RNA from wild-type cells, drug-exposed wild-type cells, modified cells, and drug exposed modified cells by guanidinium thiocyanate ***lysis***, thus disrupting the cell wall (column 25, line 64 to column 26, line 6). In Example 1, Friend et al. teaches lysing yeast cells in a phenol/chloroform/SDS buffer (column 41, lines 34-40).

In contrast, Eberwine teaches away from the lysis methods used by Friend et al. Instead, Eberwine teaches amplification via dialysis in a ***live cell*** because "it is extremely difficult to isolate RNA from a single cell" due to "the tendency of RNA to non-specifically interact with plastic and glass" (column 2, lines 63-67). Eberwine teaches that their procedure allows "the components that direct cDNA synthesis" to be brought "into immediate contact with the mRNA in a self-contained environment" (column 4, lines 27-33). Lysing the cells of Eberwine as taught by Friend et al. would render inoperative any method of Eberwine to examine the expression of a single cell.

Finally, one of skill in the art would have no incentive to obtain genetic material from a single yeast cell. A skilled yeast geneticist would instead grow an entire culture of identical yeast cells for analysis. Such cultures are routine, fast, and yield large amounts of genetic material. Eberwine, however, teaches that it is common to "experimentally manipulate single cells using whole cell and patch clamp techniques" in neurophysiological research, and that analyzing a single cell is desired over the use of brain slices, dissected tissue samples, and heterogeneous populations of cells grown in culture (column 1, lines 33-58).

Thus, the combination of Friend et al. and Eberwine fails to render Applicants' claimed invention obvious. Accordingly, Applicants respectfully request that the rejection of claims 1-3, 5, 9, 14, 18, 29-33, 35-37, 39 and 43 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

III. Claims 12, 15-17, 19-22, 24, 28 and 34 Are Patentable Over Friend et al. in View of Eberwine in Further View of Lockhart et al.

At page 4, section 2 of the instant Office Action, claims 12, 15-17, 19-22, 24, 27, 28 and 34 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al., U.S. Patent No. 6,203,987 in view of Eberwine, U.S. Patent No. 5,514,545, in further view of Lockhart et al.,

Nature Biotechnology 14:1675 (1996). The Examiner states that it would have been *prima facie* obvious to apply Lockhart et al.'s array to Friend et al.'s method of expression monitoring and classification in order to classify the many different genes in the human body simultaneously. Applicants respectfully traverse the rejection.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. As discussed above, one of skill in the art could not combine Friend et al. and Eberwine to arrive at Applicants' invention. Lockhart et al. fails to cure the deficiencies of the primary references. Accordingly, Applicants respectfully request that rejection of claims 12, 15-17, 19-22, 24, 27, 28 and 34 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

IV. Claims 4, 7, 8, 10, 11, 13 and 41 Are Patentable Over Friend et al. in View of Eberwine in Further View of Hampson et al.

At page 5, section 3 of the instant Office Action, claims 4, 7, 8, 10, 11, 13 and 41 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Hampson et al., U.S. Patent No. 6,066,457. The Examiner states that it would have been *prima facie* obvious to apply Hampson et al.'s method of producing short cDNA molecules to Friend et al.'s differential expression in order to obtain a representative sample of original mRNAs for accurate gene expression. Applicants respectfully traverse the rejection.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. As discussed above, one of skill in the art could not combine Friend et al. and Eberwine to arrive at Applicants' invention. Hampson et al. fails to cure the deficiencies of the primary references. Accordingly, Applicants respectfully request that rejection of claims 4, 7, 8, 10, 11, 13 and 41 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

V. Claim 6 Is Patentable Over Friend et al. in View of Eberwine in Further View of Van Ness et al.

At page 6, section 4 of the instant Office Action, claim 6 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Van Ness et al., U.S. Patent No. 6,248,521. The Examiner states that it would have been *prima facie* obvious to apply van Ness et al.'s partial cDNA sequences to Friend et al.'s array of Friend et al. in order to directly compare the expression of different cell types. Applicants respectfully traverse the rejection.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. As discussed above, one of skill in the art could not combine Friend et al. and Eberwine to arrive at Applicants' invention. Van Ness et al. fails to cure the deficiencies of the primary references. Accordingly, Applicants respectfully request that the rejection of claim 6 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

VI. Claims 23, 45 and 46 Are Patentable Over Friend et al. in View of Eberwine in Further View of Hampson et al. and North et al.

At page 7, section 5 of the instant Office Action, claims 23, 45 and 46 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Hampson et al. and North et al., U.S. Patent No. 6,114,502. The Examiner states that it would have been *prima facie* obvious to study the expression of North et al.'s TULP sequences in Friend et al.'s array in order to identify the cells that express the sequences for diagnostic and therapeutic purposes. Applicants respectfully traverse the rejection.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. As discussed above, one of skill in the art could not combine Friend et al. and Eberwine to arrive at Applicants' invention. Hampson et al. and North et al. fail to cure the

deficiencies of the primary references. Accordingly, Applicants respectfully request that the rejection of claims 23, 45 and 46 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

VII. Claims 38, 40 and 42 Are Patentable Over Friend et al. in View of Eberwine in Further View of Dale

At page 7 section 6 of the instant Office Action, claims 38, 40 and 42 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Dale, U.S. Patent No. 6,087,112. The Examiner states that it would have been *prima facie* obvious to study cell differentiation as taught by Dale with the array of Friend et al. in order to identify the expressed genes during differentiation or apoptosis. Applicants respectfully traverse the rejection.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. As discussed above, one of skill in the art could not combine Friend et al. and Eberwine to arrive at Applicants' invention. Dale fails to cure the deficiencies of the primary references. Accordingly, Applicants respectfully request that the rejection of claims 38, 40 and 42 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

VIII. Claim 44 Is Patentable Over Friend et al. in View of Eberwine in Further View of Hampson et al. and North et al. and Dale

At page 8, section 7 of the instant Office Action, claim 44 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Hampson et al. and North et al. and Dale. The Examiner states that it would have been *prima facie* obvious to study cell differentiation as taught by Dale with the array of Friend et al. in order to identify the expressed genes during differentiation as related to neuronal expression of

TULP proteins as they were involved in many defects as taught by North et al. Applicants respectfully traverse the rejection.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. As discussed above, one of skill in the art could not combine Friend et al. and Eberwine to arrive at Applicants' invention. Hampson et al., North et al. and Dale fail to cure the deficiencies of the primary references. Accordingly, Applicants respectfully request that the rejection of claim 44 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

IX. Claims 25 and 26 Are Patentable Over Friend et al. in View of Eberwine in Further View of Scanlon

At page 9, section 8 of the instant Office Action, claims 25 and 26 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Friend et al. in view of Eberwine in further view of Scanlon, U.S. Patent No. 5,814,489. The Examiner states that it would have been *prima facie* obvious to amplify, cleave and end label fragments with the assay of Friend et al. in order to detect and identify specific mRNAs for gene expression monitoring. Applicants respectfully traverse the rejection.

Applicants' claims are directed to novel methods of monitoring gene expression in fewer than 1000 cells. As discussed above, one of skill in the art could not combine Friend et al. and Eberwine to arrive at Applicants' invention. Scanlon fails to cure the deficiencies of the primary references. Accordingly, Applicants respectfully request that the rejection of claims 25 and 26 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

X. Conclusion

Having addressed all outstanding issues, Applicants respectfully request entry and consideration of the foregoing amendments and reconsideration and allowance of the case. To the extent the Examiner believes that it would facilitate allowance of the case, the Examiner is requested to telephone the undersigned at the number below.

Respectfully submitted,

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